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CHAPTER 24

Epitopes of HLA-A, B, C, DR, DQ, DP and MICA Antigens

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HLA antibodies in the sera of patients are a contraindication for transplantation. It is therefore essential that the specificities of HLA antibodies be correctly determined. Most HLA antibodies target specific epitopes that are shared by HLA antigens—which explains why antibodies have multiple HLA antigen specificities, including donor-specific and non-donor-specific antigens. Similarly, the antibodies to rare HLA antigens that are observed at high frequency in the sera of transplant and transfusion patients target epitopes shared with more common antigens. It is important to define these epitopes in transplant sera because they *may* be the transplantation antigens responsible for antibody-mediated graft rejection. Other antibodies detected in normal male sera and cord blood are defined as *natural* antibodies that target a different set of epitopes (1, 2). The clinical relevance of these epitopes that are targeted by natural antibodies is not yet known. However, since naturally occurring antibodies are also found in pre-transplant and multi-transfused patients (3, 4)—and are unavoidably detected alongside anti-HLA alloantibodies—their specificities have to be differentiated from those of the alloantibodies.

For our studies, single Antigen (SA) beads bearing single HLA or MICA antigens were used to show that monoclonal antibodies, alloantisera, or alloantibodies that are absorbed, then eluted, from recombinant single antigen cell lines show positive reaction to one or more HLA or MICA antigen because the antigens share the same epitope. Each epitope is defined by one or more amino acids at specific exposed locations on the surface of the HLA or MICA antigens. Epitopes targeted by naturally occurring antibodies are defined by one or more amino

acids that may be either hidden or exposed on the surface of the HLA antigens, and are designated, respectively, as *cryptic* or *exposed* epitopes.

We previously defined 103 HLA-A, B & C epitopes, as well as 60 HLA-DR, 18 HLA-DQ, 5 HLA-DP and 7 MICA epitopes (5-11). In addition, we defined 96 epitopes targeted by naturally occurring HLA antibodies that are found in the sera of normal healthy males who had no history of deliberate alloimmunizations (2, 12). Recently, we identified an additional seven HLA-A & B epitopes using murine monoclonal antibodies.

This chapter provides comprehensive lists of all epitopes defined to date. They include 110 class I, 83 class II, 7 MICA, and 96 epitopes of naturally occurring class I antibodies. Several figures show examples of epitopes on HLA and MICA molecules.

MATERIALS AND METHODS

Monoclonal Antibodies, Alloantibodies and Absorption Eluates

Murine monoclonal antibodies (mAbs) were tested at dilutions of 1:10 to 1:500,000. Anti-HLA alloantibody samples were obtained from multiparous women, from placentas, and from patients who had undergone platelet transfusions or organ transplants. Some sera were adsorbed using HLA-A, B or C recombinant HLA (rHLA) SA cell lines and HLA-DR, DQ or DP B-lymphoblast cell lines (One Lambda Inc. Canoga Park CA, USA). For studies of naturally occurring antibodies, we used sera from Mexican (MX) and Japanese (JP) normal donors and Italian cord blood (CB) donors.

Single Antigen Beads Assays and Data Analysis

Monoclonal antibodies—or antibody eluates—were examined using LabScreen® beads. The HLA alleles represented in these products are listed in Table 1. LABScreen® assays, using LABScreen® beads LS1A01, LS1A02, LS1A03, LS1A04, LS2A01 or LSMICA001, were performed according to the manufacturer's protocol. Data generated from the LABScan™ 100 were analyzed using computer software. Trimmed mean fluorescence values for the SA bead reactions were obtained from the output (.csv) file generated by the flow analyzer, and were adjusted for background signal using values of negative control sera. For HLA class I, the adjusted reaction values were then normalized using the results of the mAb W6/32 that was tested with the SA beads. All adjusted and/or normalized reactions above zero were considered potential positive reactions. However, none of the positive reactions presented here were below 500 MFI (6). To test for naturally occurring antibodies in normal male sera, class I SA beads were treated with ImmunoPure IgG Elution Buffer (EB) (Pierce, Rockford, IL, USA, Catalog 21004) to dissociate the beta-2-microglobulin (β 2m) and the peptide, then were blocked with 2% BSA (2).

HLA Amino Acid Sequences, Epitopes, and Distances Between Residues

Amino acid sequences of the HLA antigens and alleles were obtained from the Anthony Nolan Internet website (13). An epitope search program was developed to identify distinguishing amino acids (aa) that were exclusively shared by the positive antigens. The program searched for one, two, three or four common unique aa positions. The results were further filtered by restricting the search to positions on the surface of the molecule that were exposed (except for the cryptic epitopes) and were within the antibody binding span estimated at 494\AA^2 ($19 \times 26\text{\AA}$) or 750\AA^2 (14, 15). Approximate distances in angstroms between two aa were calculated

using the Cn3D structure viewer software (16) and the 3D structure of an HLA-A0201 molecule 1QEW (Orth, et al., *MMDB: Entrez's 3D-structure database* on NCBI website) (17)). Epitope ID numbers for HLA class I monoclonal and alloantibodies were assigned depending on the number of unique aa sites involved: 1-200 for one aa, 201-400 for two aa, and 401-500 for three or four aa positions. For class I epitopes of the natural antibodies, epitope ID numbers 5001-6000 were used. For MICA epitopes we used 6001–7000. For class II, we used 1001-2000 for HLA-DR, 2001-3000 for HLA-DQ, and 4001-5000 for HLA-DP.

RESULTS

HLA Class I Epitopes

Luminex beads—each coated with different rHLA SA (Table 1)—were used to identify a total of 110 HLA-A, B & C epitopes (Table 2), 47 of which were recognized by a series of monoclonal antibodies, 63 by allosera. Of the 110, 34 were HLA-A, 44 HLA-B, 4 HLA-C, 20 inter-locus HLA-A & B, 5 inter-locus HLA-B & C, and 3 inter-locus HLA-A, B & C antigen epitopes. Thirty-eight epitopes can be defined by a single aa at a single position, and all others only by a combination of two-to-four aa. Specifically, 53 epitopes were defined by two amino acids, 17 epitopes by three amino acids and 2 epitopes by four amino acids. The combination aa positions were not contiguous, but were within a conformational distance that allowed antibody binding. For most epitopes, there were one or more alternatives of a single aa or aa combinations that may define the epitope. It was beyond the scope of the assays used in our studies to precisely determine the actual amino acid(s) that bind to the antibody.

Table 3 lists the epitopes defined for each of the HLA-A, B or C individual antigen. The number of epitopes varied between 6 and 19 per antigen. For example, HLA-A1 antigen has 11 epitopes (1, 12, 13, 14, 15, 16, 208, 238, 241, 242, & 248), HLA-B7 has 19 epitopes (7, 20, 25, 32, 33, 205, 216, 222, 223, 224, 229, 231, 233, 234, 235, 401, 402, 408 & 410), and HLA-Cw4 antigen has 4 epitopes (32, 205, 232 & 244).

Table 1. Single rHLA class I antigens coated on beads used for immuno-binding assays.

Antigen	Allele								
A1	A0101	A43	A4301	B4005	B4005	B57	B5703	B81	B8101
A11	A1101	A66	A6601	B41	B4101	B58	B5801	B82	B8201
A11	A1102	A66	A6602	B41	B4102	B59	B5901		
A2	A0201	A68	A6801	B42	B4201	B60	B4001	Cw1	Cw0102
A2	A0203	A68	A6802	B44	B4402	B61	B4002	Cw2	Cw0202
A2	A0206	A69	A6901	B44	B4403	B61	B4006	Cw4	Cw0401
A23	A2301	A74	A7401	B45	B4501	B62	B1501	Cw4	Cw0403
A24	A2402	A80	A8001	B46	B4601	B63	B1516	Cw5	Cw0501
A24	A2403	A34	A3402	B47	B4701	B64	B1401	Cw6	Cw0602
A25	A2501	A36	A3601	B48	B4801	B65	B1402	Cw7	Cw0702
A26	A2601			B49	B4901	B67	B6701	Cw8	Cw0801
A29	A2901	B13	B1301	B50	B5001	B7	B0702	Cw12	Cw1203
A29	A2902	B13	B1302	B51	B5101	B71	B1510	Cw14	Cw1402
A3	A0301	B18	B1801	B51	B5102	B72	B1503	Cw15	Cw1502
A30	A3001	B27	B2705	B52	B5201	B73	B7301	Cw16	Cw1601
A31	A3101	B27	B2708	B53	B5301	B75	B1502	Cw17	Cw1701
A30	A3002	B35	B3501	B54	B5401	B75	B1511	Cw18	Cw1802
A32	A3201	B37	B3701	B55	B5501	B76	B1512	Cw9	Cw0303
A33	A3301	B38	B3801	B55	B5502	B77	B1513	Cw10	Cw0302
A33	A3303	B39	B3901	B56	B5601	B78	B7801	Cw10	Cw0304
A34	A3401	B39	B3905	B57	B5701	B8	B0801		

Table 2. One hundred ten HLA class I epitopes.

Epitope # Assigned	SA beads reactive with monoclonal or eluted antibody ^a	Position and unique aa for possible epitope Sites ^b	Alloserum or mAbs	rHLA Cells used for adsorption
1	A1,36	44K/150V/158V/	Z3945.OL	N/A (mAb)
2	A2,69	107W	W6090.LO	N/A (mAb)
3	A23,24	65G	Z1238.TO	N/A (mAb)
4	A25,26,34,43,66	(9Y)+149T/(74D)+149T	Z8855.TO	N/A (mAb)
5	A29,43	62L	X5518.TO	N/A (mAb)
6	A3	161D	X8341.EO	N/A (mAb)
7	B7,8,13,18,27,35,37,38,39,4005,41,42,44,45,46,47,48,49,50,51,52,53,54,55,56,59,60,61,62,64,65,67,71,72,73,75,76,77,78,81,82	65Q	F1398-2EH1	N/A (mAb)
8	B13	145L/ 41T+46A	Z0693.TO	N/A (mAb)
9	B38,39,67	158T	Z7567.RO	N/A (mAb)
10	B46	46A+66K [69R] ^c	Z5550.DM	N/A (mAb)
11	B8	(67F)+131R/ (67F)+177D/ (67F)+180E [9D] ^c	X7768.TO	N/A (mAb)
12	A1,11,25,26,43,6601	163R	AS264	A2501
13	A1,2,3,11,24,36,68,69,80	144K	W7252.AO	A0101
14	A1,23,2402,80,B76	166D/167G	X9288.OO	A2402
15	A1,26,29,36,43,80	76A	X9288.OO	A2902
16	A1,36,11,25,26,34,43,6601,80,B73	[90D] ^c	W6409	A8001
17	A2,B57,58	62G	X4632	B5801
18	A2,68,69	142T/145H	X4632	A6901
19	A2,23,24,68,69	127K	Z6303	A6901
20	B7,8,41,42,48,60,81	177D/180E	ARC JA	B0702
21	B13,4005,41,44,45,47,49,50,60,61	41T	W7248.BO	B4402
22	B8,13,18,35,37,38,39,3905,4005,41,4102,44,4403,45,47,48,49,50,51,52,53,59,60,61,62,64,65,71,72,75,76,77,78	69T	Z7227.00	B1301
23	A23,24,25,32,B38,49,51,52,53,57,58,59,63,77	80I	RGF19	B4901

Table 2 cont'd.

Epitope # Assigned	SA beads reactive with monoclonal or eluted antibody ^a	Position and unique aa for possible epitope Sites ^b	Alloserum or mAbs	rHLA Cells used for adsorption
24	A23,24,25,32,B13,2705,37,38,44, 47,49,51,52,53,57,58,59,63,77	82L/83R	RGF19/ W8822.AO	B4403/ A3201
25	B7,8,18,2708,35,39,4005,41,42,45,46,48,50,54, 55,56,60,61,62,64,65,67,71,72,73,75,76,78,81,82	[80N] ^c	X1779.BO	B5501
27	A203,25,26,34,43,66	149T	S33S	nn
28	A23,24,80	62E	Z5571.I0	N/A (mAb)
29	A80	56E /62E+65R/62E+76/ 144K+151R/ 163E+166D/ 163E+167G	2004-08361	nn
30	A1102	19K	2002-05676 / 2002-00048	nn
31	A30,31	56R	2002-01731	nn
32	A2,3,11,2403,25,26,29,30,31,32, 33, 34,36,43, 66,68,,69,74,B7,8, 13,18, 27,35,37,38,39,4005,41, 42,46,47, 48,49,50,51,52,53,54, 55,56,57,58, 59,60,61,62,63,64, 65,67,71,72,73, 75,77,78,81, Cw1,2,4,5,6,7,8, 9,10, 12,14,15, 16,17,18	167W	FS32-5D8B6	N/A (mAb)
33	A32,74,B7,8,13,18,27,35,37,38,39,4005,41,42,44, 45,46,47,48, 49,50,51,52,53,54,55,56,57,58,59,60, 61,62,63,64,65,67,71,72, 73,75,76,77,78,81,82	[109L] ^c	F2164-4A7A3	N/A (mAb)
35	B18,35,37,51,52,53,78,58	45T	FN3814- 3E1G4	N/A (mAb)
36	A30	17S / 56R+73T	2005-02583	nn
37	Cw7	194L	A132	Cw0702
38	A2,A25,A26,A29, A31,A32, A33, A34, A43,A66,A68,A69,A74, B73, Cw7,Cw17	253Q	A39 / A40	A2501 / A0201
39	Cw2,9,10,15	21H	Z7921.00 / A113	Cw0202 / Cw0303(Cw9)
40	Cw5,8	177K	A129	nn
41	B73,Cw7,17	267Q	A61	Cw1701
43	B13, B46,B57,B62, B63, B75 B76,B77	46A	Z3633.T0	N/A (mAb)
201	A2	43Q+62G/ 62G+66K/ 62G+76V/ 62G+79G	S8043	N/A (mAb)
202	A23	65G+151R/ 127K+144Q/ 127K+151R	Z1060.TO	N/A (mAb)
203	A2402	156Q+166D/ 156Q+167G	Z1022.HO	N/A (mAb)
204	A32,74,B8,18,37,38,39,41,42,54,55, 59,64,65,67	[109L+163T] ^c	F760-5B5D8	N/A (mAb)
205	A32,74,B7,8,4005,41,42,48,60,61,73,81,Cw1,2,4, 5,6,7,8, 9,10,12,14,15,16,17,18	109L+131R	F1119-9F4E7	N/A (mAb)
206	A36	158V+163T/ 158V+166E/ 158V+167W	X7138.HO	N/A (mAb)
207	B57,58,63	43P+65R/ 65R+163L/ 66N+131S/ 66N+163L	Z1203.TO	N/A (mAb)
208	A1,3,11,24,36,80	142I+144K/ 144K+145R	X9174	A8001
209	A11,25,26,43,6601	163R+166E/ 158A+163R/ 163R+167W	Z2076.@O	A2501
210	A2,3,11,68,69	76V+144K	SE896	A1101
211	A203,25,26,34,43,66,B46,62,76	[(152E+156W)] ^c	RGF40	B76 (B1512)
212	A23,24,32,B38,49,51,52,53,57, 58, 59,63, 77	[80I+90A/ 80I+149A] ^c	Z8059.C0	B5701
213	A23,25,26,29,30,31,32,33,34,43, 66, 74	138M+144Q	Z6895.00	A3101
214	A25,26,33,34,66,68,69	43Q+62R/ 62R+109F	Z3044	A6901
215	A33,34,68,69,B8,18,37,38,39,41, 42, 54,55,59,64,65,67	62R+163T	Z7227.00	B5401/A6801
216	B7,8,18,2708,35,39,4005,41,42,45,48,50,54,55, 56,60,61,62,64, 65,67,71,72,73,75,76,78,81,82	76E+80N/ 76E+82R/ 76E+83G	Z3038	B4102
217	B13,2705,37,44,47	76E+80T/ 79R+80T/ 80T+82L/ 80T+83R	RGF41 / Z6895.00	B4403 / B2705
218	B13,57,62,63,75,76,77	46A+76E	X9733	B76 (B1512)
219	B18,35,37,51,52,53,78	45T+62R/ 45T+65Q/ 45T+66I/ 45T+69T/ 45T+71T	X6101	B1801
220	A3301,B18,51,52,64,65,78	90A+(171H)	SE897	A3301

Table 2 cont'd.

Epitope # Assigned	SA beads reactive with monoclonal or eluted antibody ^a	Position and unique aa for possible epitope Sites ^b	Alloserum or mAbs	rHLA Cells used for adsorption
221	B35,4005,46,49,50,51,52,53,56, 57,58, 62,63,71,72,75,77,78	163L+167W	RGF19 (mAb: FV3306-1A1A3)	B5601
222	A6602,B7,13,27,47,48,60,61,73, 81, Cw2,Cw17	163E+166E/ 163E+167W	A4	Cw0202
223	B7,13,27,47,48,60,61,81	76E+163E	RGF37	B0703
224	B7,27,42,54,55,56,57,58,63,67, 73, 81,82	43P+69A	X0786.00	B5801/ B5502
225	B8,59	(67F)+163T	RGF38	B0801
226	B8,18,37,38,39,41,42,54,55,59, 64, 65,67	66I+163T	Z6895.00	B1801
227	B8,18,35,39,4005,41,45,48,50, 60,61,62,64,65,71,72,75,76,78	69T+80N/ 69T+ 82R/ 69T+83G	X4715.D0	B4501
228	B18,37,38,39,54,55,59,64,65,67	131S+163T	Z6331.00	B3905
229	B7,27,42,54,55,56,67,73,81,82	65Q+69A	Z6331.00	B8101
230	B38,49,51,52,53,59,77	65Q+80I/ 69T+ 80I	Z2980.B0	B5201 + others
231	B7,48,81	41A+178K	S35C	N/A
232	B54,55,59,Cw1,4,5,6,7,8,12,14, 15,16,18	(103L)+163T	Z2611.R0	N/A (mAb)
233	A25,32,B7,8,13,18,27,35,37,38,39,4005,41,42,44, 45, 46,47,48, 49, 50,51,52,53,54,55,56,57,58,59, 60,61,62,63,64,65, 67,71,72, 73,75,76,77,78,81,82	[79R +127N] ^c	F352-10F9E1	N/A (mAb)
234	B7,42,46,54,55,56,67,81,82	[43P+(70Q) / 65Q+(70Q)] ^c	AS627	N/A
235	B27,47,61,7,48,60,73,81,13	138T+163E	X7021.E0	N/A (mAb)
236	B57,58	43P+62G/ 41A+43P+62G 17R+41A+43P+ 62G / 19E+41A+43P+ 62G	Z1173, N0 / F667-2E1E4/ 2005-01035 (Allo)	N/A (mAb)
237	B57,63 (weak B58)	46A+65R / 41A+46A+65R	X8442.E0	N/A (mAb)
238	A1,2,3,11,25,26,29,32,33,34,36, 43, 66,68,69,74,B57,58,63	56G+65R	FG2755-11A3D7	N/A (mAb)
239	B46,73	43P+76V/ 65Q+76V/ 76V+79R/ [76V+80N 41+43P+76V/ 41+65Q+76V/ 73T+76V+79R/ 73T+76V+80N] ^c	2002-00142	nn
240	B76	163L+166D/ 163L+167G	0-4 / 38991/ 38995/ 38998/ 39002	nn
241	A1,11,25,26,34,36,43,6601,80	65R+90D / 43Q+90D / 90D+138M	AS264	A3601
242	A1,2,3,1101,26,29,30,31,33, 34,36, 43,66,68,69,74,80	19E+79G	0544HA	N/A (mAb)
243	A25,26,A33,34,66,68,69,B63	62R+65R	FC1043-6B4D1B9 (Eluted from A2501 rHLA cell line)	N/A (mAb)
244	Cw2,4,5,6,15,17,18	80K	A113	Cw1701
245	B35,4005,46,49,50,51,52,53,56,57,58,62,63,71, 72,75,77,78, Cw9, Cw10	163L+167W	A6	B62 (B1501) / B35
246	B46,73,Cw1,7,8,9,10,12,14,16	76V+80N / 73T+76V+79R	Z9016.00 / 2002-00142	Cw1802/ nn
247	A2, 3,11,24,25,26,29, 30,31, 33,34,36,43,66,68,69	109F+166E/ 109F+167W	0131HA3-7	N/A (mAb)
248	A1,3,11,36	62Q+151H	X8342.H0	N/A (mAb)
249	A23,24,25,32,B2705,37,38 44,47, 49,51,52,53, 57,58,59 63,77(B13 Negative)	82L+145R/ 83R+145R	0473HA	N/A (mAb)
250	A23,A24,A32,B13,2705,37,38,44,47,49,51,52,53, 57,58,59,63,77(A25 Negative)	82L+90A/ 83R+90A	548HA1-4	N/A (mAb)
401	B7,27,42,54,55,56,57, 58,63,67, 81, 82	43P+69A+76E	Z6328.00	B0702
402	B7,42,54,55,56,67,81,82	65Q+69A+(70Q)	Z9009.A0	B8201
403	B46,62,75,76,77	41A+46A+65Q	Z6845.B0	B76(B1512)
404	A11	149+150A+163R/149+158A+163R 149+163R+166E/149+163R+167W	2002-00323	nn
406	B2705	65Q+69A+ 80T 65Q+69A+82L 65Q+69A+83R	X4221.DQ/ X3494.N0	N/A(mAb)
407	A24	127K+142I+144K/ 127K+142I+151H/ 127K+144K+145R/ 127K+145R+151H	X8598.T0 / S8036	N/A(mAb)
408	B7	(147W)+163E+177D (147W)+163E+180E	Z5754.R0	N/A(mAb)

Table 2 cont'd.

Epitope # Assigned	SA beads reactive with monoclonal or eluted antibody ^a	Position and unique aa for possible epitope Sites ^b	Alloserum or mAbs	rHLA Cells used for adsorption
409	B63	43P+62R+65R	Z6856.PK	N/A(mAb)
410	B7,42,54,55,56,67,81,82	[41A+46E+67Y/43P+46E+67Y 43P+46E+ 70Q] ^c [43P+69A+70Q/43P+70Q+76E [46E+65Q+67Y/46E+65Q+ 70Q] ^c	2002-05252	nn
411	B2708	(63E)+69A+80N/(70K)+76E+80N (70K)+76E+82R/(70K)+76E+83G (70K)+80N+131S	2005-04739	nn
412	A0201	(9F)+142T+149A / (9F)+145H+149A	2002-00245/ 2004-08903	nn
414	B49,52,63	62R+(63E)+80I	2002-01799	nn
415	B46,57,58,63	[(63E)+(71A)+ 163L] ^c	F633-3E8H4	N/A (mAb)
417	A11,B57,58	[(9Y)+41A+ (63E)+(95I)] ^c	F698-4F9F9	N/A (mAb)
418	A26,B13	(32Q)+62R+ (77N)+80T/ (45M)+62R+ (77N)+80T/	X6954.TO	N/A (mAb)
419	B49,51,52,63,77	80I+90A+ 127N+(152E)/ 80I+109L+ 131S+(152E)/ 82L+90A+ 127N+(152E)/ 83R+90A+ 127N+(152E)	FC2121- 5A4A1	N/A (mAb)
420	B8,B64,B65	69T+(74D)+ 158A+163T	FS964-1A3B4	N/A (mAb)
421	B46,Cw1,8,9,10,14,16	(73T)+76V+ 80N+90A	A6	Cw0102
422	A0201,0206,3,11,24,A68,69	149A+150A+ 151H	X8032.H0	N/A (mAb)
423	A23,25,32, B2705,37,38,44,47,49,51,52,53,57, 58,59, 63,77 (A24, B13 Negative)	83R +144Q+ 145R	Z1153	N/A (mAb)

Columns, left to right, show: Epitope #; HLA antigens exclusively sharing the epitope, aa (including alternatives) that define the epitope; serum or monoclonal antibody, rHLA cell line used for absorption and elution of antibodies. SA = Single Antigen. nn = Absorption not needed because all positive antigens for the sera shared the epitope. N/A= Not applicable

^a Alleles are designated only when other alleles of the same antigen did not react with the eluate.

^b Possible alternative epitopes are separated by "/". Epitopes that are defined by more than a single position/ aa are separated by "+". Amino acids that are not exposed at the surface of the HLA molecule are between parentheses.

^c Epitope also shared by C-locus antigens based on aa sequences but not proven by antibody test with SA beads are in square brackets.

Table 3. Epitopes on HLA class I antigens.

Antigen	# of Epitopes	Epitopes
A1	11	1 12 13 14 15 16 208 238 241 242 248
A11	16	12 13 16 30 32 208 209 210 238 241 242 247 248 404 417 422
A2	16	2 13 17 18 19 27 32 38 201 210 211 238 242 247 412 422
A23	12	3 14 19 23 24 28 202 212 213 249 250 423
A24	16	3 13 14 19 23 24 28 32 203 208 212 247 249 250 407 422
A25	19	4 12 16 23 24 27 32 38 209 211 213 214 233 238 241 243 247 249 423
A26	17	4 12 15 16 27 32 38 209 211 213 214 238 241 242 243 247 418
A29	8	5 15 32 38 213 238 242 247
A3	10	6 13 32 208 210 238 242 247 248 422
A30	6	31 32 36 213 242 247
A31	6	31 32 38 213 242 247
A32	14	23 24 32 33 38 204 205 212 213 233 238 249 250 423
A33	10	32 38 213 214 215 220 238 242 243 247
A34	14	4 16 27 32 38 211 213 214 215 238 241 242 243 247
A36	12	1 13 15 16 32 206 208 238 241 242 247 248
A43	14	4 5 12 15 16 27 32 38 209 211 213 238 241 242
A66	16	4 12 16 27 32 38 209 211 213 214 222 238 241 242 243 247
A68	13	13 18 19 32 38 210 214 215 238 242 243 247 422
A69	14	2 13 18 19 32 38 210 214 215 238 242 243 247 422
A74	8	32 33 38 204 205 213 238 242
A80	9	13 14 15 16 28 29 208 241 242
B13	16	7 8 21 22 24 32 33 43 217 218 222 223 233 235 250 418
B18	15	7 22 25 32 33 35 204 215 216 219 220 226 227 228 233

Table 3 cont'd.

In general, there was no correlation between the number of epitopes identified for the antigen and the antigen's frequency in the population. For example, HLA-A2, which is the most frequent antigen in the population (30%-54%), has 16 epitopes while A25, with a frequency of 0-6%—if it is present—has 19 epitopes.

Certain aa residue positions on the HLA antigen were found more frequently in the epitope definitions than others. The most frequent position was 163, located in the $\alpha 2$ domain of the heavy chain and well exposed on the surface of the molecule. In contrast, position 161—which is one aa away from position 163, and equally exposed—defines only one epitope (Fig. 1). In addition, certain individual aa were observed more frequently than others in defining epitopes. For example, threonine (T) was the most frequent aa while cysteine (C) was the

least frequent (Fig. 2). Length of the side chain—and whether the aa were acidic, basic, hydrophobic, or hydrophilic—did not correlate with the frequency at which the aa were found in epitopes.

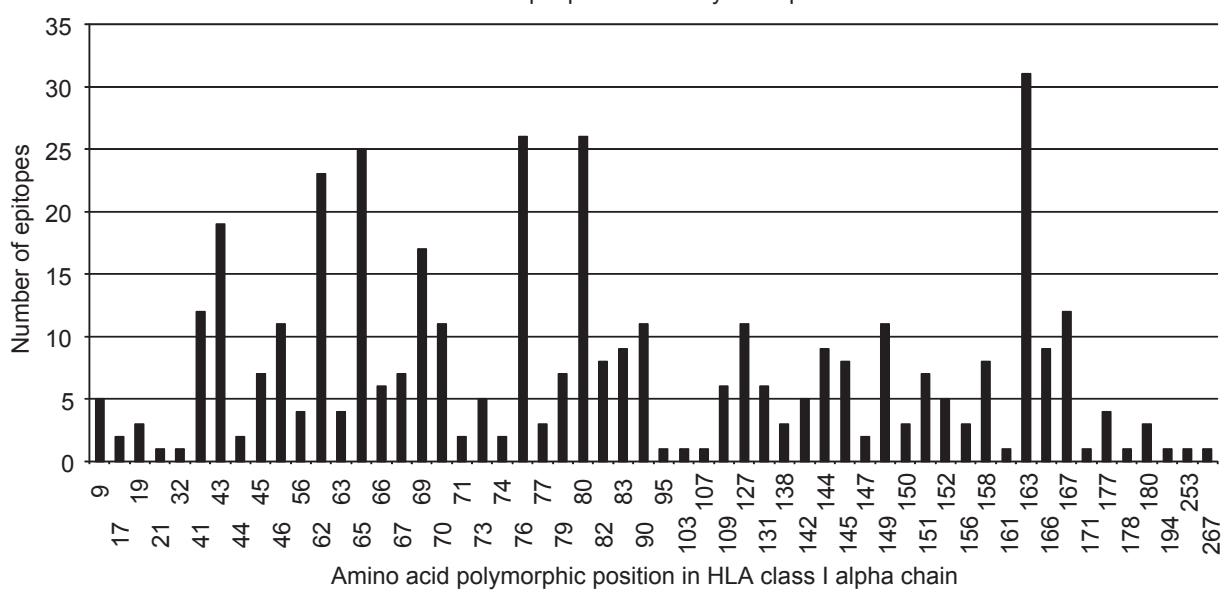
Table 4 shows the number of epitopes defined for each HLA locus or inter-locus HLA-A, B & C antigens. The number of aa that define the epitopes are also shown. For example, 23 epitopes were shared exclusively among B-locus antigens, and each epitope was defined by a combination of two aa.

Figures 3-10 are examples of HLA class I epitopes. All positive reactions in each example are reactions of the SA beads with a monoclonal antibody, diluted allosera or eluted alloantibodies. Amino acid(s) shared exclusively among the positive antigens define the epitope. The 3D structure of the HLA antigens shows the likely location of each epitope.

Figure 1.

Polymorphic amino acid positions on HLA class I antigens

Number of epitopes defined by each position

**Figure 1.**

The frequencies at which polymorphic position of amino acids appear in the definition of the HLA class I epitopes are shown in this figure. For

example, the most frequent position was 163. This defines more than 30 epitopes while position 161, only one amino acid away, defines only one epitope.

Figure 2.

Amino acids were found at different frequencies in defining the HLA class I antigens epitopes. The amino acid threonine (T), was found in 373 of all the epitope alternative sites for the 110 class I epitopes identified. On the other hand, cysteine (C) was found only once.

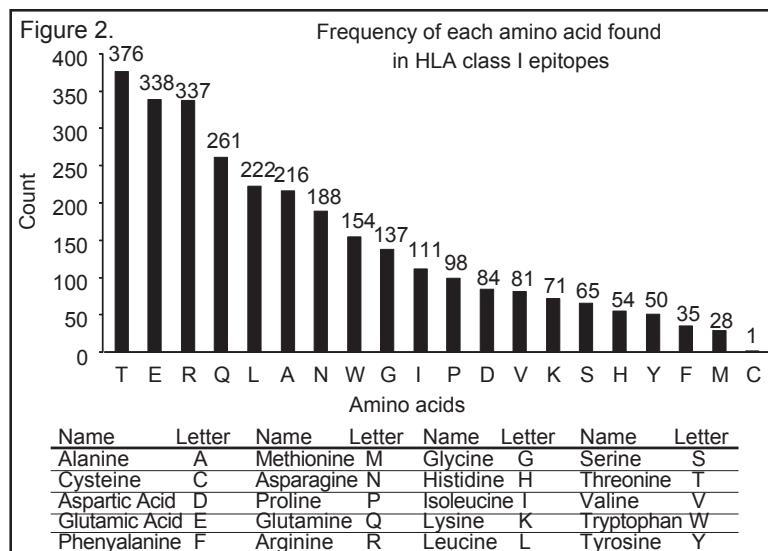


Table 4. Distribution of 110 HLA class I epitopes among the HLA ABC loci. Also shown the number of amino acids that define the epitopes.

Number of Epitopes	23	17	14	10	9	10	6	3	3	4	2	2	3	2	1	1
HLA - Locus	B	A	A	B	B	AB	AB	C	BC	A	BC	ABC	AB	B	ABC	C
Number of aa defining epitope	2	1	2	3	1	2	1	1	2	3	1	2	3	4	1	2

Figure 3.

Antibody eluted from the A2501 rHLA SA cell line reacts strongly with only A11, A25, A26, A43, and A6601 SA beads. These antigens have the unique combination of arginine (R) at position 163 and glutamic Acid (E) at position 166 on the HLA molecule (epitope #209, Table 2).

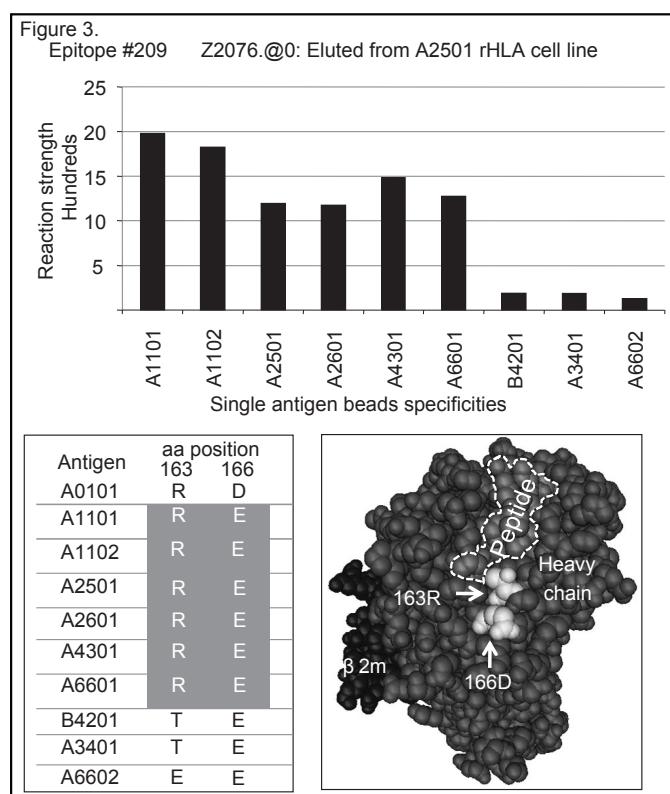
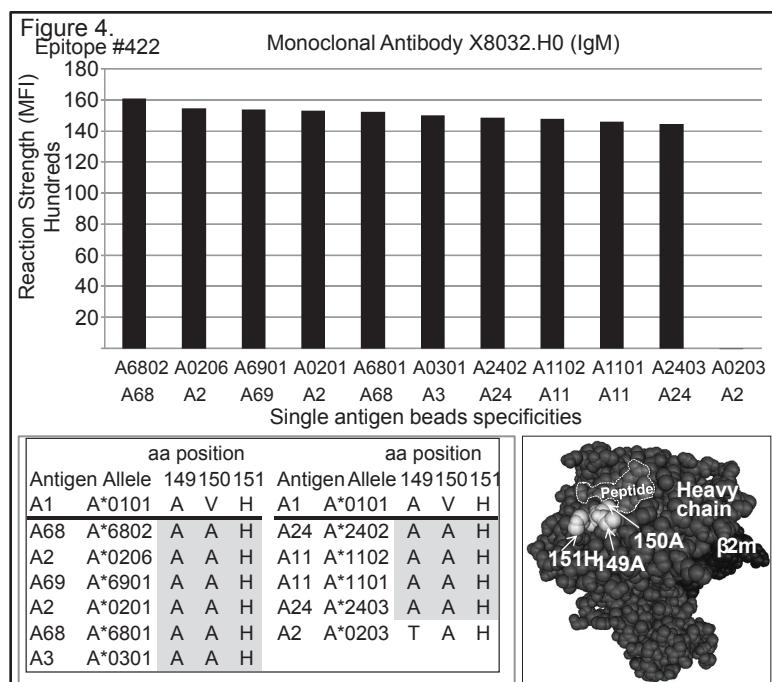


Figure 4.

Monoclonal antibody X8032.H0 reacts with A-locus HLA antigens sharing the amino acids alanine (A), alanine (A) and histidine (H) at positions 149, 150 and 151 respectively. All three aa combined define epitope 422 (Table 2). Although this mAb reacts positive with A0201 and A0206 it is negative with A0203 which has the aa threonine (T) instead of alanine at 149.

**Figure 5.**

SA beads B13, B4005, B41, B44, B45, B47, B49, B50, B60, & B61 were positive with alloantibody eluted from a B4402 rHLA SA cell line. The positive antigens all share the amino acid threonine (T) at position 41 on a side loop of the HLA molecule (epitope #21, Table 2).

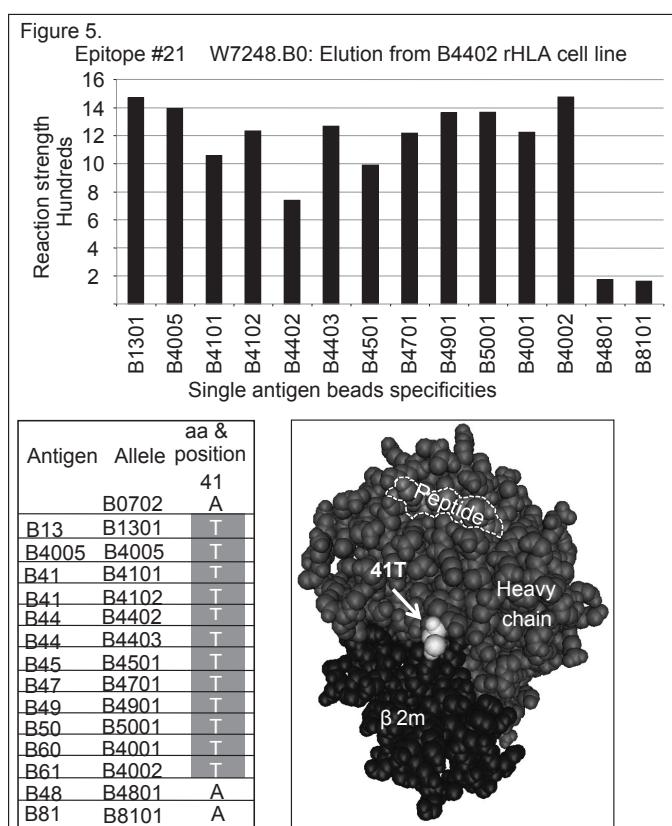
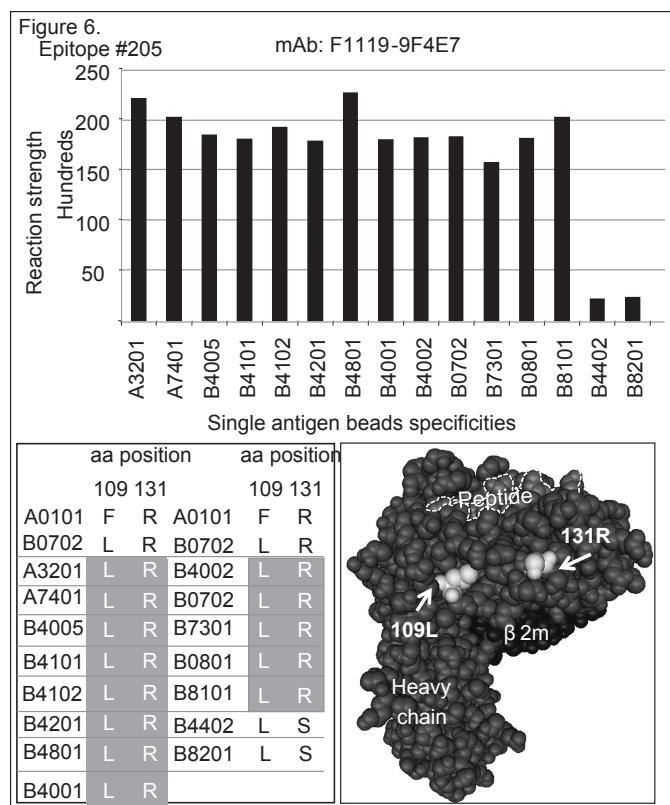


Figure 6.

Single antigens A32, A74, B4005, B41, B42, B48, B4001(60), B4002(61), B7, B73, B8, and B81 exhibit strong immuno-binding with mAb F1119-9F4E7, and share unique amino acid leucine (L) at position 109, and arginine (R) at position 131 (epitope #205, Table 2). The two amino acids are approximately 15 Å apart and are located on the side loops of the HLA molecule.

**Figure 7.**

SA beads Cw8 and Cw5 were positive with alloantibody A129. Both positive antigens share exclusively the amino acid lysine (K) at position 177 located in the alpha 2 domain of the HLA heavy chain molecule (Epitope 40, Table 2).

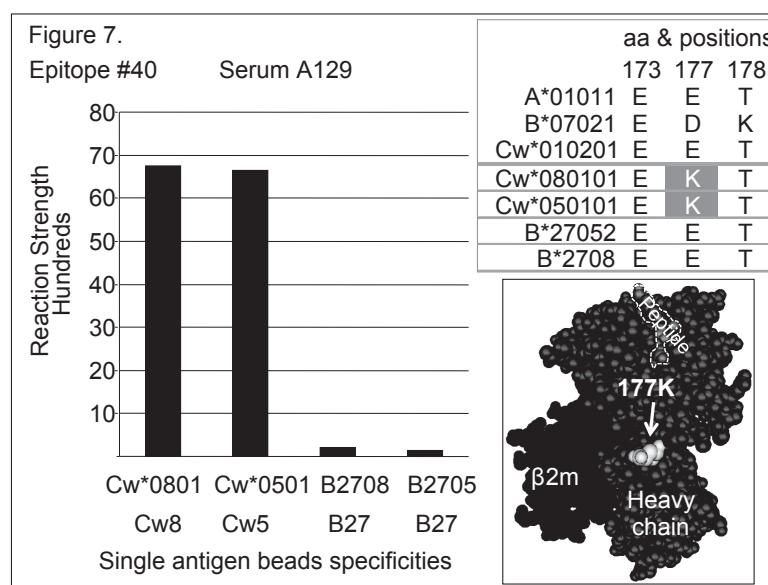
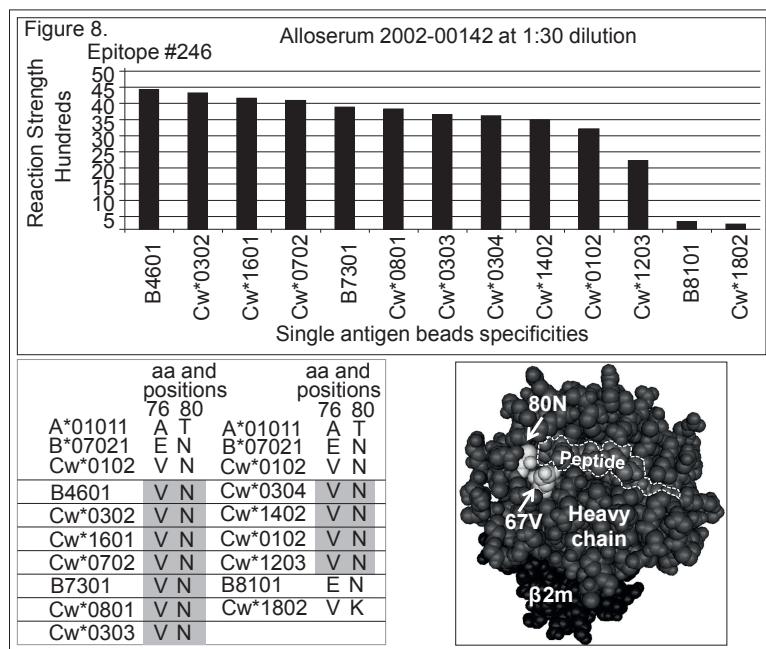


Figure 8.

Example of an alloserum positive with B and C antigens all sharing a unique epitope. Antigens, B46, B73 and Cw0302 (Cw10), Cw1601, Cw0702, Cw0801, Cw0303 (Cw9), Cw0304 (Cw10), Cw1402, Cw0102 and Cw1203 share the aa valine (V) at position 76 and asparagine (N) at position 80. Both aa are located in the alpha 1 domain of the HLA antigen and combined define the epitope. (Epitope 246, Table 2).

**Figure 9.**

Example of an inter-locus epitope shared by A, B, and C class I antigens. Antigens, A3303, A6801, Cw0702, Cw1701, A7401, A3301, A6901, A6802, A3401, A2901, A2902, A2501, A0203, A2601, A3101, A3201, A0201, A6601, A4301, A6602, A0206, and B7301 shared the amino acid glutamine (Q) at position 253. Although glutamine at this position is located close to the cell membrane and may not be easily accessible to the antibody, it is the only aa exclusively shared by the positive antigens (Epitope 38, Table 2).

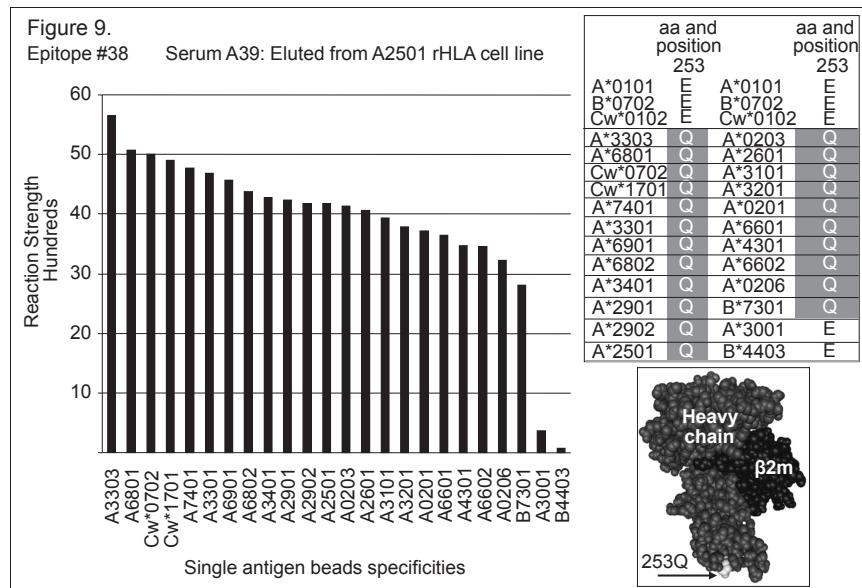
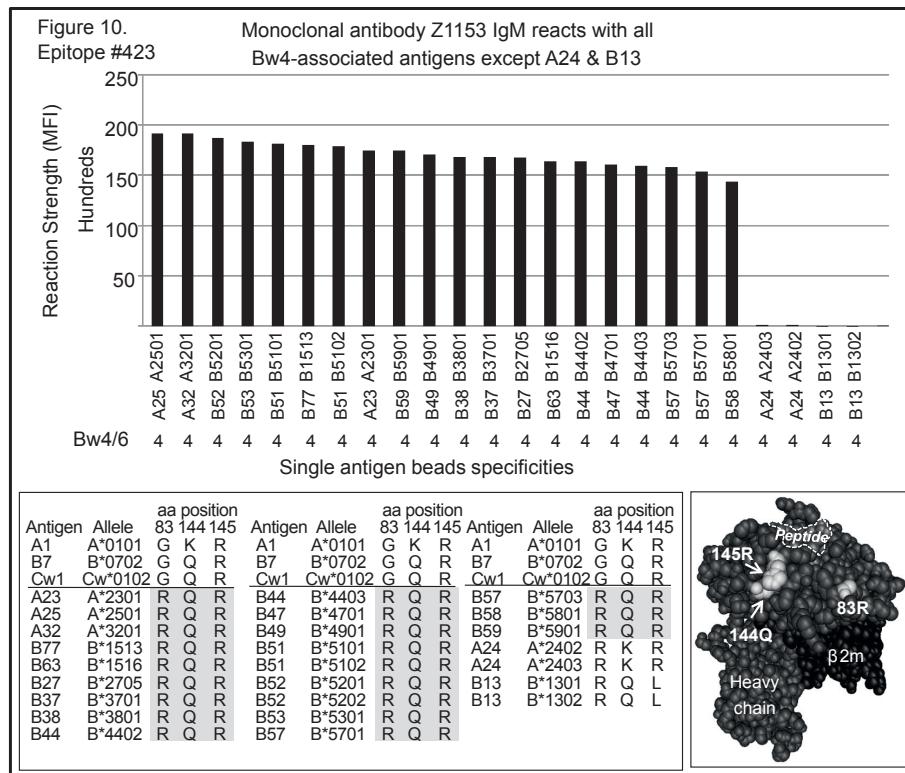


Figure 10.

Anti-Bw4 mAb Z1153 recognizes epitope 423 (Table 2) which is defined by the aa arginine (R), glutamine (Q), and arginine (R) at positions 83,144, and 145, respectively, and is shared by all Bw4-associated antigens except A2402, A2403, B1301 and B1302.



Epitopes of Natural HLA Class I Antibodies Found in the Sera of Normal Healthy Males and in Cord Blood

Almost 60% of all epitopes identified for naturally occurring antibodies were private epitopes, found only on single antigens. The rest were public epitopes, targeted by multi-specific antibodies. Fifty-eight epitopes (60%) were accessible for antibody binding only on the dissociated HLA antigens (heavy chains). Of these, 41 were defined by hidden aa and therefore designated cryptic epitopes. Thirteen were defined by at least one hidden aa in addition to exposed aa, so are designated partially cryptic. For four epitopes, no hidden aa were found to define them and therefore they were designated exposed epitopes. All hidden aa are listed within parentheses and are believed to be inaccessible in the intact form of the antigens.

Table 5 lists 58 mostly cryptic epitopes that are accessible only on the dissociated forms of one HLA antigen—for example, HLA antigen A2 (epitope 5001), or B37 (epitope 5016). Other epitopes are shared by the dissociated forms of two or more antigens—for example, A23,A24 (epitope 5002),

B7,B42,B54,B55,B56,B67,B81,B82 (epitope 5024) or Cw4,Cw6,Cw17,Cw18 (epitope 5037). One-to-four aa define the epitopes. In most cases, more than one alternative aa can define the epitope. However, for space considerations we list only the epitope defined by the least number of aa that are separated by distances not exceeding the antibody's binding span. Antibodies that target the most frequently occurring epitopes were found in all three sera groups, each from a different ethnic population. For instance, A-locus epitope 5007 was found in 22 sera (19 MX, 2 JP, 1 CB). B-locus epitope 5024 was found in 12 sera (8 MX, 3 JP, 1 CB), epitope 5031 found in 17 sera (13 MX, 4 JP). C-locus epitope 5036 was found in 18 sera (9 MX, 9 JP).

Table 6 lists 38 epitopes accessible on intact HLA class I antigens. Thirty-two epitopes were defined by exposed aa while six epitopes were defined by two or more aa, of which only one was hidden. All 38 epitopes were either unique to a single HLA antigen or were shared by a group of two or more intact antigens. The most frequently occurring epitopes—30, 5064, 5072, 5073, 5074, 5077 and 5084—were found, respectively, on antigens A1102 (23 sera), A3002 (23 sera), B63 (11 sera),

Table 5. Fifty-eight mostly cryptic epitopes located on dissociated class I HLA antigen -- heavy chains.

Epitope # assigned ^a	Dissociated antigen(s) with distinct epitope ^b	Possible epitope site ^c	Epitope Descr. ^d	Ag. form ^e	MX ^f	JP ^f	CB ^f	TOTAL
5001	A2	(74H)	C	D	4	1		5
5002	A23,A24	(9S)+(70H)	C	D	3			3
5003	A25,A26,A33,A3303,A34,A66,A6602,A68,A6802,A69	(63N)+(67V)	C	D	1			1
5004	A2901 & A2902	(9T)+(114R)	C	D	1	1		2
5005	A29,A43	(63Q)	C	D	5			5
5006	A3002	(152R)	C	D	3			3
5007	A31,A33	(73I)	C	D	19	2	1	22
5008	A3401	(63N)+(66K)	C	D	1	2		3
5009	A3402	(63N)+(66K)+(156L)	C	D		1		1
5010	A80	(31S)	C	D	14	4	1	19
5011	B2705,B2708	(97N)	C	D	1			1
5012	B27,B44,B47	(24T)+(116D)	C	D	1			1
5013	B2705	(45E)+(77D)	C	D	3			3
5014	B2708	(70K)+(77S)	C	D	4			4
5015	B27,B37	(9H)+(77D)	C	D	1			1
5016	B37	(99S)+(116F)	C	D	11	1	1	13
5017	B37,B47	(70N)+(77D)	C	D	1			1
5018	B4402	(77N)+(156D)	C	D	3	1		4
5019	B5501	(97T)+(116L)+(152E)	C	D	1	1		2
5020	B57,B58,B63	(70S)	C	D		1		1
5021	B65	(11A)+(97W)	C	D	3	1		4
5022	B67	(70Q)+(116F)	C	D			1	1
5023	B7,B48,B60,B81	(178K)	C	D	1			1
5024	B7,B42,B54,B55,B56,B67, B81,B82	(66I)+(70Q)	C	D	8	3	1	12
5025	B72	(45E)+(77S)+(116S)	C	D	2			2
5026	B75	(67S)+(77S)+(95I)	C	D	2			2
5027	B8	(9D)	C	D	6	3		9
5028	B8,B37,B42,B82	(24S)+(156D)	C	D	3			3
5029	B8,B42,B82	(45E)+(156D)	C	D	2	1		3
5030	B8,B42,B37,B41,B4402, B45,B82	(156D)	C	D	1			1
5031	B82	(24S)+(99F)	C	D	13	4		17
5032	Cw1	(6K)	C	D	1			1
5033	Cw2	(211T)	C	D	6			6
5034	Cw1502	(1C) +(116L)	C	D	4			4
5035	Cw16	(116S)+(156Q)	C	D	4			4
5036	Cw17	(116F)+(143S)	C	D	9	9		18
5037	Cw4,Cw6,Cw17,Cw18	(73A)+(77N)	C	D	3		1	4
5038	Cw6	(9D)+(97W)	C	D	5	1		6
5039	Cw7	(66K)+(99S)	C	D	2			2
5040	Cw8	(152T)	C	D	3			3
5041	A1,A36	(67M)+(70H)	C	D	3		1	4
5042	A0203	(114H)+149T	PC	D	4	2		6
5043	A2501	(81A)+90D	PC	D	1			1
5044	A1,A26,A36,A29,A43	(74D)+76A	PC	D	2			2
5045	A1,A3,A11,A30,A31,A32, A36,A74,A80	(63E)+71S+(95I)	PC	D	3			3
4	A25,A26,A34,A43,A66	(9Y)+149T	PC	D	8	1		9
5047	A203,A25,A26,A34,A43, A66	(152E)+184A	PC	D	4	2		6
5048	A23,A24,A34	43Q+66K+(74D)	PC	D	1			1
5049	A6602	(114Q)+163E	PC	D	2			2
5050	A7401	66N+(77D)+109L	PC	D	1			1
5051	B4403	(156L)+167S	PC	D	1			1
5052	B76	(70N)+166D	PC	D	1	1		2
5053	A1,A3,A11,A30,A31,A32, A36,A74	62Q	PC	D	2			2
17	A2,B57,B58	62G	PC	D		1		1
5055	B44,B45,B82	167S	E	D	4			4
5056	A25,A26,A29,A31,A32, A33, A34,A43,A66,A74	246S	E	D	1			1
5057	A2,A203,A206,A25,A26, A29, A32,A34,A43,A66, A6602, A68,A6802,A69,A74	184A	E	D		1		1
5058	A2,A203,A206,A25,A26,A29,A31,A32,A33, A3303, A34, A43,A66,A6602,A68,A6802, A69,A74	193A	E	D	1			1

^a Epitopes 4 &17 have been assigned to alloant bodies (Table 2). ^b one or more dissociated HLA class I antigens that share a unique epitope. ^c Amino acids and their positions on the HLA dissociated antigens define each epitope. Hidden amino acid positions are between parentheses. Epitopes that are defined by more than a single position/aa are separated by "+". Although there are alternative definitions, for space considerations we list here only the most probable definitions based on distances among the amino acids and the orientations of their side chains. ^d C=cryptic, PC=partially cryptic, and E=exposed epitopes. These designations are in reference to the intact HLA antigen. ^e D=dissociated HLA antigens (heavy chain of the HLA class I antigens). ^f MX=Mexican, JP=Japanese, CB=cord blood.

Table 6. Thirty-eight mostly exposed epitopes located on intact HLA class I antigen.

Epitope # assigned ^a	Dissociated antigen(s) with distinct epitope ^b	Possible epitope site ^c	Epitope Descr. ^d	Ag. form ^e	MX ^f	JP ^f	CB ^f	TOTAL
5059	A0101	158V+163R	E	I		1		1
1	A1,A36	44K	E	I			1	1
30	A1102	19K	E	I	6	13	4	23
201	A2	43Q+62G	E	I			1	1
19	A2,A23,A24,A68,A69	127K	E	I			1	1
3	A23,A24	65G	E	I	2			2
5060	A2403	65G+166E	E	I	1	1		2
5061	A2501	76E+149T	E	I	2	1		3
5062	A2601	62R+76A	E	I	1	1		2
214	A25,A26,A33,A3303,A34, A66, A6602, A68,A6802,A69	43Q+62R	E	I	1			1
5063	A2901 & A2902	62L+163T	E	I	1	1		2
31	A30,31	56R	E	I	2			2
5064	A3002	17S+76E	E	I	23			23
5065	A36	150V+163T	E	I		1		1
5066	A6602	149T	E	I	5	2		7
5067	A6901	66N+107W	E	I		2		2
5068	A80	56E+	E	I	4	2		6
5069	A32,B57,B58,A25,B63	65R+76E	E	I		1		1
406	B2705	65Q+69A+80T	E	I	1			1
236	B57,B58	43P+62G	E	I	3			3
5070	B57,B63	46A+65R	E	I	1			1
5071	B60	41T+147L	E	I	1			1
5072	B63	43P+62R+65R	E	I	10	1		11
5073	B76	163L+166D	E	I	6	4		10
5074	B82	162D	E	I	5	7		12
5075	Cw*0102,0302,0303,0304,1402,1802	219W	E	I		1		1
5076	Cw16	193L	E	I	1			1
5077	Cw17	170G	E	I	6	6		12
5078	Cw7	273S	E	I	1	1		2
5079	Cw8	16S+90D	E	I		1		1
5080	Cw9	91R	E	I	3			3
5081	Cw9,Cw10	163L+173K	E	I	2	2		4
203	A2402	(156Q)+166D	PC	I	1	1		2
5082	A2901	62L+(102H)+163T	PC	I		1		1
5083	A3401	43Q+(66K)+90D	PC	I	3	2		5
5084	B4501	(9H)+167S	PC	I	7	5		12
5085	B8	(67F)+131R	PC	I	3			3
5086	Cw6	80K+90D+(114D)	PC	I	1			1

^a Epitopes #1,30,201,19,3,214,31,406,236, 203 have been assigned to alloantibodies (Table 2). ^b one or more dissociated HLA class I antigen(s) that share a unique epitope. ^c Amino acids and their positions on the HLA dissociated antigens define each epitope. Hidden amino acid positions are between parentheses. Epitopes that are defined by more than a single position/aa are separated by "+". Although there are alternative definitions, for space considerations, we list here only the most probable definitions based on distances among the amino acids and the orientations of their side chains. ^d E=exposed and PC=partially cryptic epitopes. These designations are in reference to the intact HLA antigen. ^e I=intact HLA antigens.(heavy chain+β2m+peptide). ^f MX=Mexican, JP=Japanese, CB=cord blood.

B76 (10 sera), B82 (12 sera), Cw17 (12 sera) and B4501 (12 sera). Several of these epitopes were identified earlier as target epitopes of alloantibodies. For example, the epitope shared by A1,A36 HLA antigens—and defined by aa 44K—was designated earlier as epitope #1 (Table 2).

Evidence for the dissociation of HLA antigens on the SA beads is shown in Figures 11 and 12. Monoclonal antibodies W6/32 and anti- β 2m—both of them positive with the intact HLA antigens—have negative reactions with the dissociated antigens. Except for a few reactions with marginal values, all of these reactions were below the 1000 MFI cutoff point.

Figures 13-15 graphically present the fluorescence data that show the positive reactions of dissociated and intact antigens for epitopes that are defined as cryptic or exposed. Only the highest negatives are included to more clearly demonstrate the marked drop in signal from the epitope-positive antigens.

Figure 13 illustrates the reactions of serum 316 with the A3101, A3303 and A3301 intact and dissociated antigens of the SA beads. It is clear from these data that the strength of reactions when

using dissociated antigens is up to 10 times greater than with intact antigens. Only A3101, A3303, and A3301 share the aa isoleucine (I) at position 73. As illustrated in the 3-D antigen figures, this position is located underneath the peptide. These findings strongly suggest that aa 73(I) defines a cryptic epitope that becomes accessible for antibody binding only when the peptide is removed. The weak reactions shown with the intact antigen beads are most likely due to their presence on the same beads with dissociated antigens produced during the purification or coupling process (epitope 5007, Table 5). Similar observations apply to eight B-locus antigens that share the cryptic epitope 5024 and four C-locus antigens that share the cryptic epitope 5037 (Figs. 14-15).

Figure 11.

W6/32 monoclonal antibody has positive reactions with intact HLA antigens (NT = non-treated) on the SA beads and negative reactions with SA beads treated with the acid elution buffer (EB) indicating antigen dissociation.

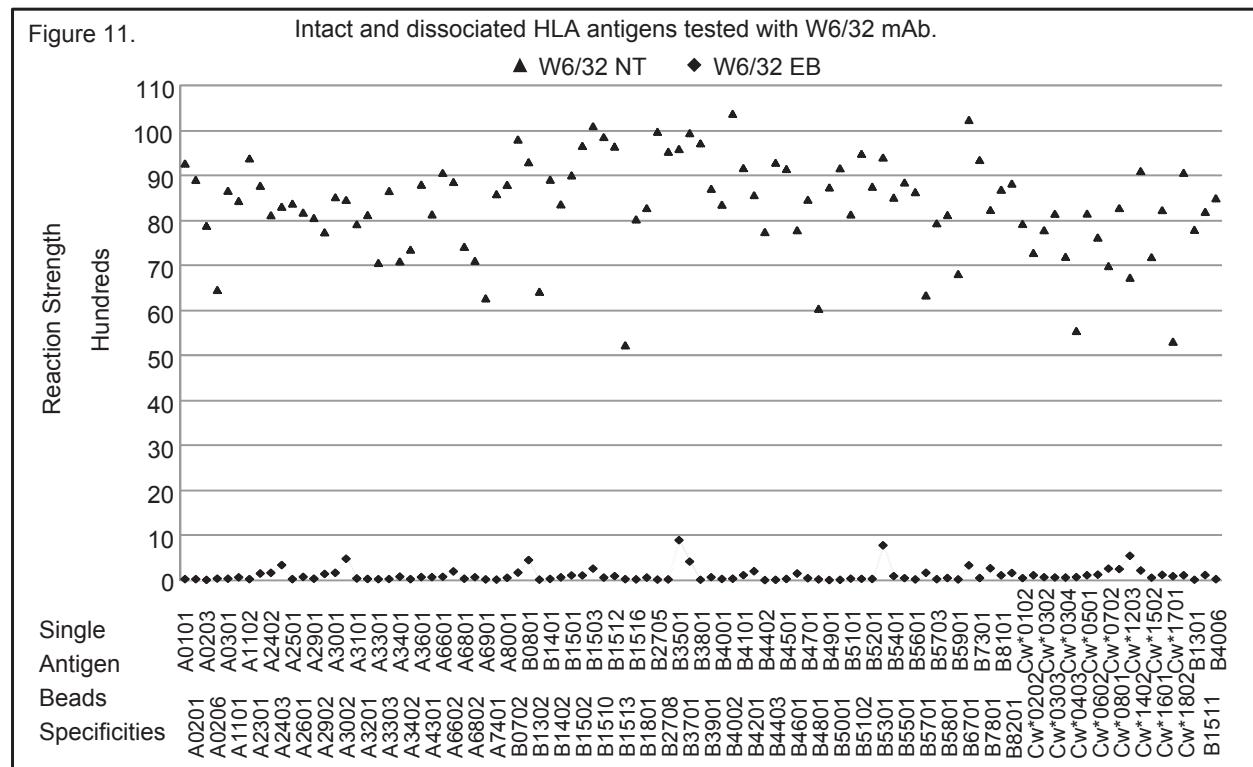


Figure 12. Intact and dissociated HLA antigens tested with β 2m mAb

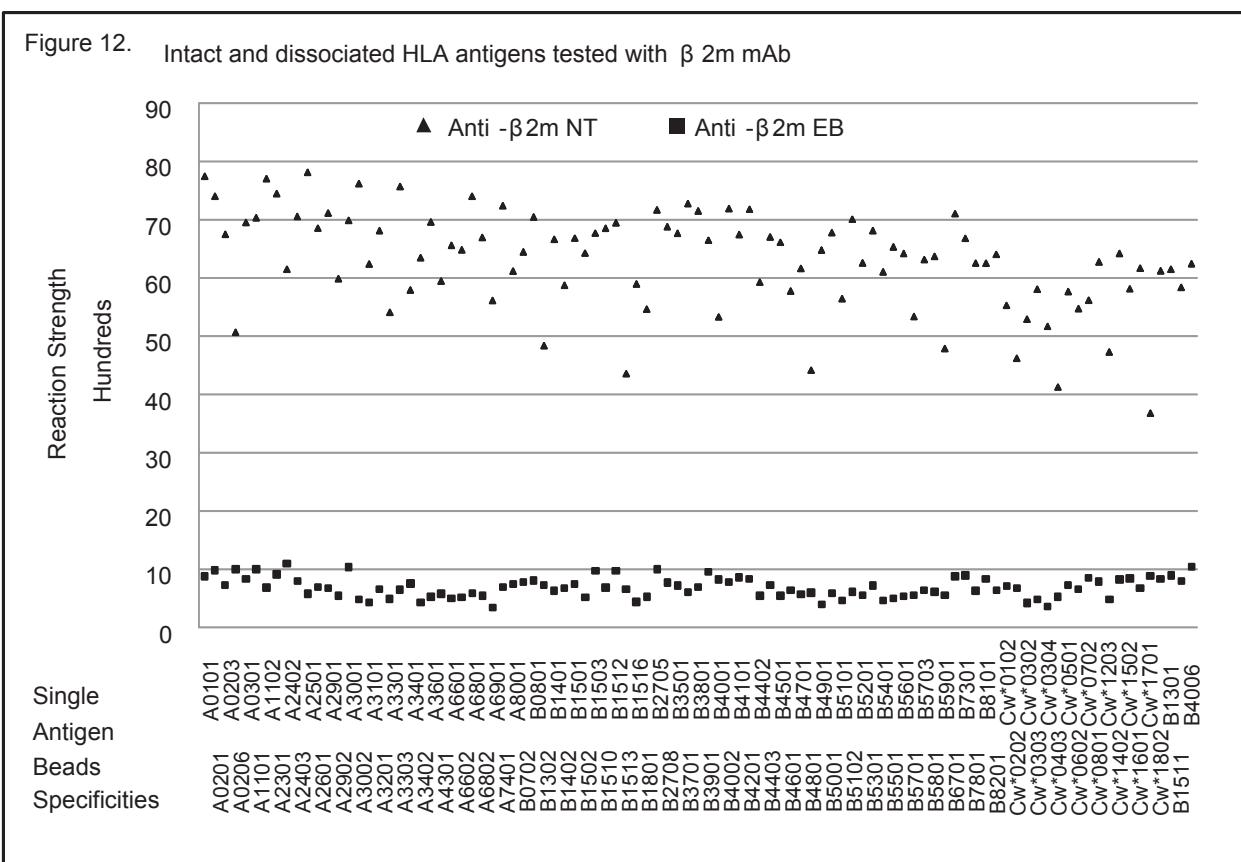


Figure 12.

Anti- β 2m monoclonal antibody has positive reactions with intact HLA antigens (NT = non-

treated) on the SA beads and negative reactions with SA beads treated with the acid elution buffer (EB) indicating antigen dissociation.

Figure 13.

Serum 316 shows weak to moderate positive reactions with intact HLA antigens A3101, A3301, A3303 and very strong positive reactions with the dissociated forms (heavy chains) of the three A-locus antigens. A3101, A3301, A3303 are the only antigens that share the amino acid isoleucine (I) at position 73. 73I lies beneath the peptide in the intact antigens and becomes fully exposed after the antigens are dissociated, resulting in stronger binding with the antibody. Isoleucine at position 73 defines a cryptic epitope on the dissociated antigens (epitope 5007, Table 5).

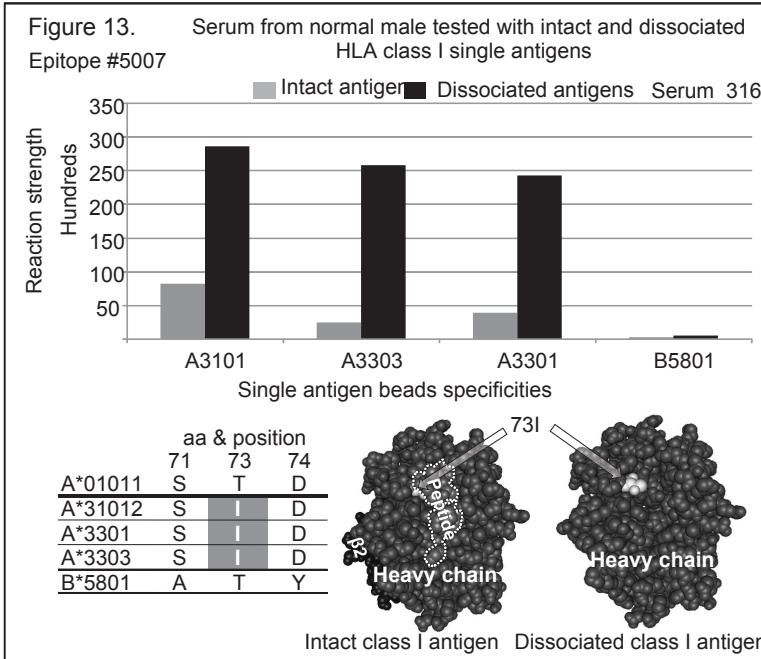
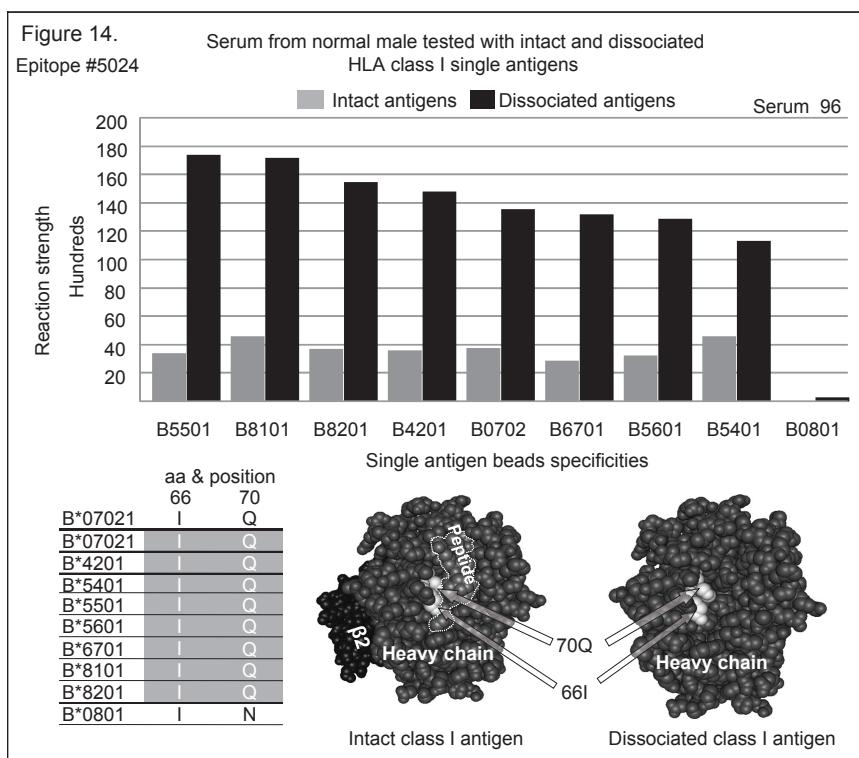
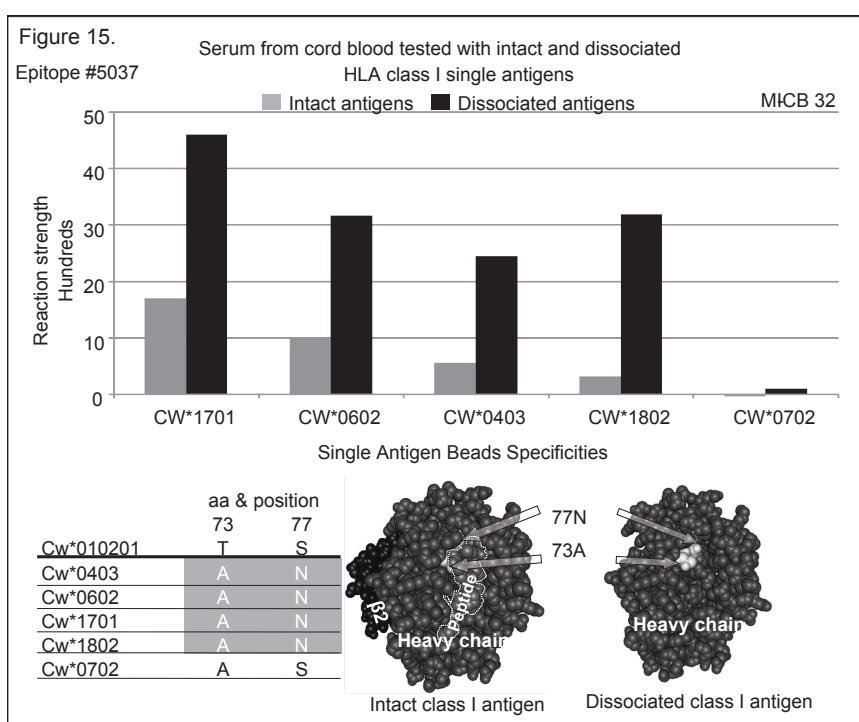


Figure 14.

Serum 96 shows weak to moderate positive reactions with intact HLA antigens B5501, B8101, B8201, B4201, B0702, B6701, B5601, and B5401 and very strong positive reactions with the dissociated forms (heavy chains) of the eight B-locus antigens. These are the only antigens that share the amino acids isoleucine (I) and glutamine (Q) at positions 66 and 70, respectively. The two positions appear to be partially exposed in the intact antigens. In the dissociated forms of the antigens, the two amino acids become fully exposed, binding strongly with the antibody. The two amino acids define a cryptic epitope (5024, Table 5).

**Figure 15.**

Serum MI-CB32 shows weak positive reactions with intact HLA antigens Cw1701, Cw0602, Cw0403, Cw1802 and moderate positive reactions with the dissociated forms (heavy chains) of the four C-locus antigens. These are the only antigens that share the amino acids alanine (A) and asparagine (N) at positions 73 and 77, respectively. The two positions are hidden beneath the peptide in the intact antigens. In dissociated antigens, the two amino acids become fully exposed, binding with moderate strength with the antibody. The two amino acids define a cryptic epitope on the dissociated antigens (epitope 5037, Table 5).



HLA Class II Epitopes

Sixty HLA-DR, 18 HLA-DQ, and 5 HLA-DP epitopes have been defined to date (Tables 7-9). Most HLA-DR epitopes were defined by one aa residue on the HLA-DR beta chain. However, DQB, DQA and DP epitopes were defined by one-to-four aa.

Table 10 shows the number of epitopes defined for each HLA-DR antigen, that number varying

from 8 to 21. For example, DR7 has 21 epitopes (1001, 1002, 1003, 1007, 1008, 1011, 1018, 1022, 1025, 1028, 1029, 1032, 1035, 1037, 1039, 1043, 1403, 1405, 1410, 1411, and 1602). Similarly, all DQ antigens have more than one epitope.

Figures 16-21 show examples of one DR, two DQB, one DQA, and two DQP epitopes.

Table 7. Sixty HLA-DR epitopes.

Epitope #	DR antigens sharing epitope	Position/ amino acid ^a	Epitope #	DR antigens sharing epitope	Position/ amino acid ^a
1001	DR7,DR9,DR53	4Q	1030	DR1,DR12	85A
1002	DR1,DR7,DR15,DR16,DR103	9W	1031	DR1,DR51,DR103	96E
1003	DR1,DR4,DR7,DR9,DR15, DR16,DR51,DR53,DR103	10Q	1032	DR7,DR8,DR9,DR10,DR11, DR12,DR13,DR14,DR17, DR18, DR52	96H
1004	DR4,DR10	11V	1033	DR10,DR15,DR16,DR53	96Q
1005	DR1,DR9,DR10	13F	1034	DR4	96Y
1006	DR11,DR14,DR15, DR17,DR18,DR52	13S	1035	DR4,DR7,DR9	98E
1007	DR7,DR51	13Y	1036	DR52	98Q
1008	DR7	25Q	1037	DR4,DR7,DR9,DR51,DR52	104A
1009	DR4,DR8,DR11,DR13,DR14, DR15,DR16,DR17	28D	1038	DR4,DR10,DR51,DR53	120N
1010	DR1,DR9,DR51,DR53	31I	1040	DR4,DR8,DR10,DR11,DR12, DR13,DR14,DR17,DR18	140T
1011	DR7,DR14	37F	1041	DR8,DR11,DR12,DR13,DR14, DR17,DR18	149H
1012	DR12	37L	1042	DR4	180L
1013	DR1,DR14,DR15,DR103	37S	1043	DR7,DR9,DR10	181M
1014	DR10,DR53	40Y	1401	DR9	9K,30G
1015	DR11,DR12,DR13, DR15,DR17	47F	1402	DR51	9Q/108T
1016	DR4,DR8,DR13	57S	1403	DR1,DR7,DR15,DR16,DR103	9W/10Q
1017	DR11	58E	1404	DR9,DR51	11D/28H
1018	DR7,DR8,DR11,DR12,DR13, DR16,DR51,DR103	70D	1405	DR7	11G/14K
1019	DR9,DR10,DR14,DR53	70R	1406	DR4	11V/13H
1020	DR15	71A	1407	DR8,DR12	13G/16Y
1021	DR4,DR13,DR17,DR18,DR52	71K	1408	DR10,DR53	38A/40Y
1022	DR7,DR17,DR18,DR52	73G	1409	DR13	57S/71K
1023	DR9,DR14,DR53	74E	1410	DR7,DR9,DR12	57V/60S
1024	DR8	74L	1411	DR4,DR7,DR9	98E/104A
1025	DR7,DR52	74Q	1601	DR8,DR11,DR12,DR13,DR14, DR17,DR18	9E/10Y/11S/ 12T/13S
1026	DR17,DR18	74R	1602	DR7	11G/14K/ 25Q/30L
1027	DR17,DR18,DR52	77N	1603	DR15,DR16	11P/13R/ 133L/142M
1028	DR1,DR4,DR7,DR9,DR10,DR 11,DR12,DR13,DR14,DR15, DR16,DR51,DR53,DR103	77T	1604	DR11,DR13,DR14, DR17,DR18	11S/12T/13S
1029	DR7,DR9	78V	1605	DR4	13H/33H/ 96Y/180L
1039	DR1,DR7,DR9,DR15,DR16, DR51,DR52,DR53,DR103	140A	1606	DR14	57A/60H/112Y

Columns, left to right, show: Epitope #; HLA-DR antigens exclusively sharing the epitope; and amino acid (including combinations) that define the epitope. Most amino acid positions listed in this table are not exposed on the surface of the molecule and therefore may not be the amino acids that bind to the antibody.

^a Possible alternative epitopes are separated by "/".

Table 8. Eighteen HLA-DQ epitopes.

Epitope # ^a	DQ antigens sharing epitope	Position/ amino acid ^b
2001	DQB2	28S/30S/37I/52L/55L
2002	DQB4	56L
2003	DQB4,DQB5,DQB6,DQB7,DQB8,DQB9	28T/46V/52P
2004	DQB5,DQB6	84E/85V/86A/89G/90I/221Q
2005	DQB7	45E
2006	DQB7,DQB8,DQB9	55P
2007	DQB4,DQB5,DQB6	52P+55R
2008	DQB2,DQB5,DQB7,DQB8,DQB9	(9Y+11F)
2009	DQB2,DQB4,DQB5,DQB6,DQB8,DQB9	34R+45G
2010	DQB4,DQB5,DQB6,DQB8,DQB9	45G+46V
2011	DQB5,DQB0601	38V+46V
2012	DQB8,DQB9	45G+55P
2013	DQB2,DQB4,DQB7,DQB8,DQB9	84Q/85L/86E/87L/89T/220H/221H
2014	DQB4,DQB7,DQB8,DQB9	77T+84Q/77T+85L/77T+86E/77T+87L/182N
2015	DQB5	70G+71A/116I/125S
2017	DQA1*0201	47K+52H+54L
2018	DQA1*04/DQA1*05/DQA1*06	40G/47C
2019	DQA1*03	26S/47Q/56R/187T

Columns, left to right, show: Epitope #; HLA-DQB, HLA-DQA antigens exclusively sharing the epitope; and amino acid (including alternatives) that define the epitope.
^a 2008 defined by mAb. ^b Possible alternative epitopes are separated by "/". Epitopes that are defined by more than a single position/aa are separated by "+". Amino acids that are not exposed at the surface of the HLA molecule are between parentheses.

Table 9. Five HLA-DP epitopes.

Epitope #	DP antigens sharing epitope	Position/ amino acid ^a
4001	DPB1*0101, DPB1*0301, DPB1*0501, DPB1*0901, DPB1*1001, DPB1*1101, DPB1*1301, DPB1*1401, DPB1*1701, DPB1*1901	84D+85E+86A+-87V
4002	DPB1*0301, DPB1*0901, DPB1*1401, DPB1*1701	55D+56E+-57D
4003	DPB1*0201, DPB1*0402, DPB1*1001, DPB1*1801	55D+56E+-57E
4004	DPB1*1101, DPB1*1501	33Q
4005	DPB1*0201, DPB1*0401, DPB1*0402	84G+85G+86P+87M

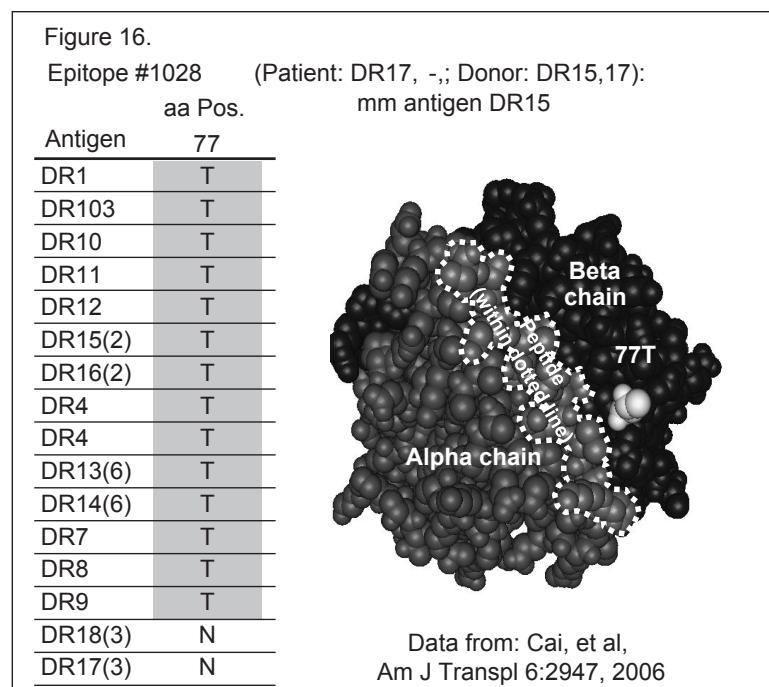
Columns, left to right, show: Epitope #, HLA-DPB antigens exclusively sharing the epitope; and amino acid or combination of amino acids that define the epitope are shown.
^a Epitopes that are defined by more than a single position/aa are separated by "+".

Table 10. Epitopes on HLA class II antigens.

Antigen	# of Epitopes	Epitopes													
DR1	10	1002	1003	1005	1010	1013	1028	1030	1031	1039	1403				
DR10	11	1004	1005	1014	1019	1028	1032	1033	1038	1040	1043	1408			
DR103	8	1002	1003	1013	1018	1028	1031	1039	1403						
DR11	11	1006	1009	1015	1017	1018	1028	1032	1040	1041	1601	1604			
DR12	11	1012	1015	1018	1028	1030	1032	1040	1041	1407	1410	1601			
DR13	12	1009	1015	1016	1018	1021	1028	1032	1040	1041	1409	1601	1604		
DR14	13	1006	1009	1011	1013	1019	1023	1028	1032	1040	1041	1601	1604	1606	
DR15	12	1002	1003	1006	1009	1013	1015	1020	1028	1033	1039	1403	1603		
DR16	9	1002	1003	1009	1018	1028	1033	1039	1403	1603					
DR17	12	1006	1009	1015	1021	1022	1026	1027	1032	1040	1041	1601	1604		
DR18	10	1006	1021	1022	1026	1027	1032	1040	1041	1601	1604				
DR4	15	1003	1004	1009	1016	1021	1028	1034	1035	1037	1038	1040	1042	1406	1411
DR51	11	1003	1007	1010	1018	1028	1031	1037	1038	1039	1402	1404			
DR52	9	1006	1021	1022	1025	1027	1032	1036	1037	1039					
DR53	11	1001	1003	1010	1014	1019	1023	1028	1033	1038	1039	1408			
DR7	21	1001	1002	1003	1007	1008	1011	1018	1022	1025	1028	1029	1032	1035	1037
		1043	1403	1405	1410	1411	1602								1039
DR8	9	1009	1016	1018	1024	1032	1040	1041	1407	1601					
DR9	17	1001	1003	1005	1010	1019	1023	1028	1029	1032	1035	1037	1039	1043	1401
		1410	1411												1404
DQA1*0201	1	2017													
DQA1*03	1	2019													
DQA1*04	1	2018													
DQ2	4	2001	2008	2009	2013										
DQ4	7	2002	2003	2007	2009	2010	2013	2014							
DQ5	8	2003	2004	2007	2008	2009	2010	2011	2015						
DQ0601	6	2003	2004	2007	2009	2010	2011								
DQ7	6	2003	2005	2006	2008	2013	2014								
DQ8	8	2003	2006	2008	2009	2010	2012	2013	2014						
DQ9	8	2003	2006	2008	2009	2010	2012	2013	2014						

Figure 16.

Example of an epitope shared by DR antigens. Antigens DR1, R103, DR10, DR11, DR12, DR15(2), DR16(2), DR4, DR13(6), DR14(6), DR7, DR8, DR9, DR18(3), and DR17(3) share the amino acid threonine (T) at position 77 of the HLA DR beta chain. Therefore threonine at this position identifies this epitope (Epitope # 1028, Table 7).

**Figure 17.**

Example of an epitope shared by DQB antigens. Antigens DQ4(0401) and DQ4(0402) share the aa leucine (L) at position 56 of the HLA DQB chain. Therefore leucine at this position identifies this epitope (Epitope # 2003, Table 8).

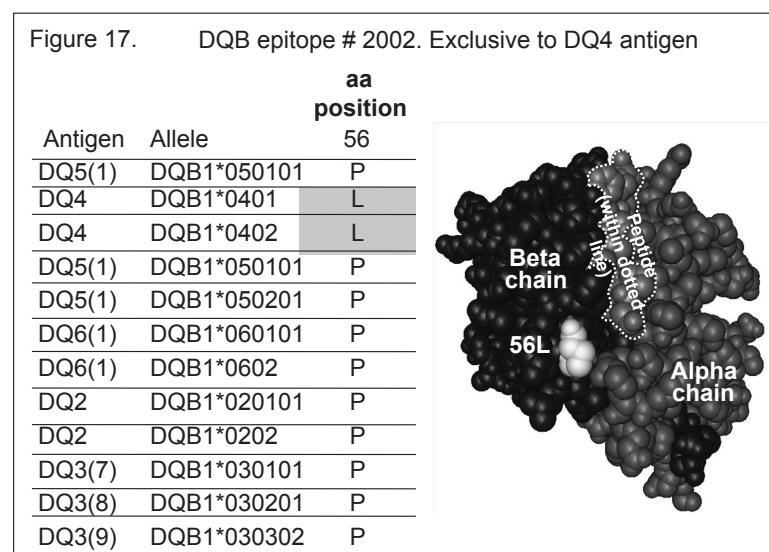
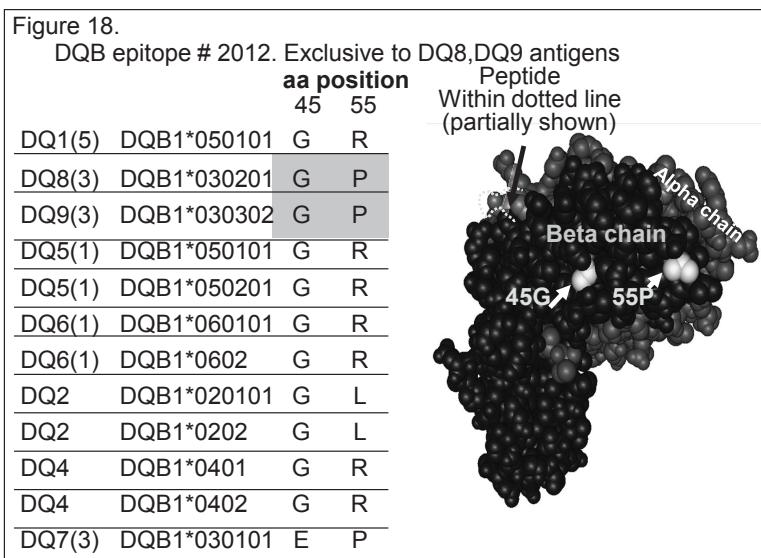
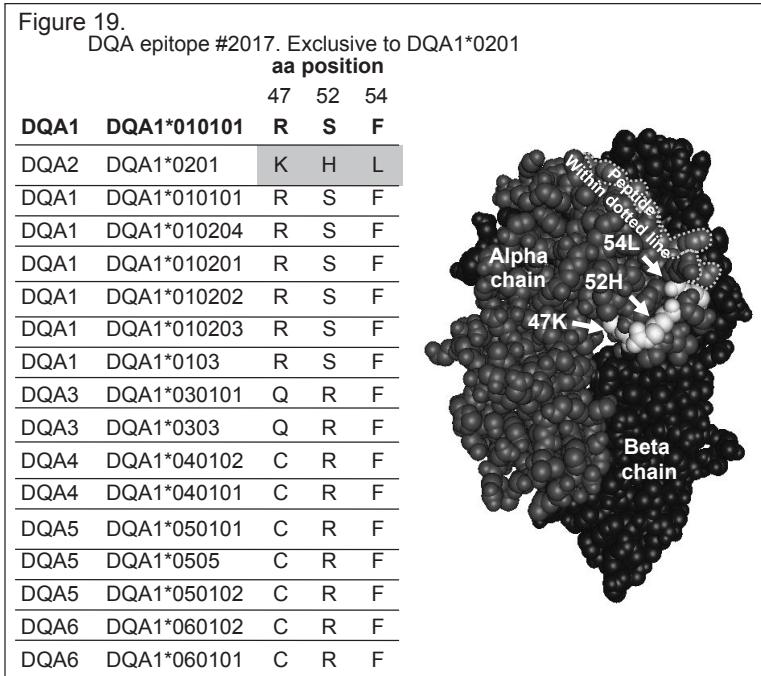


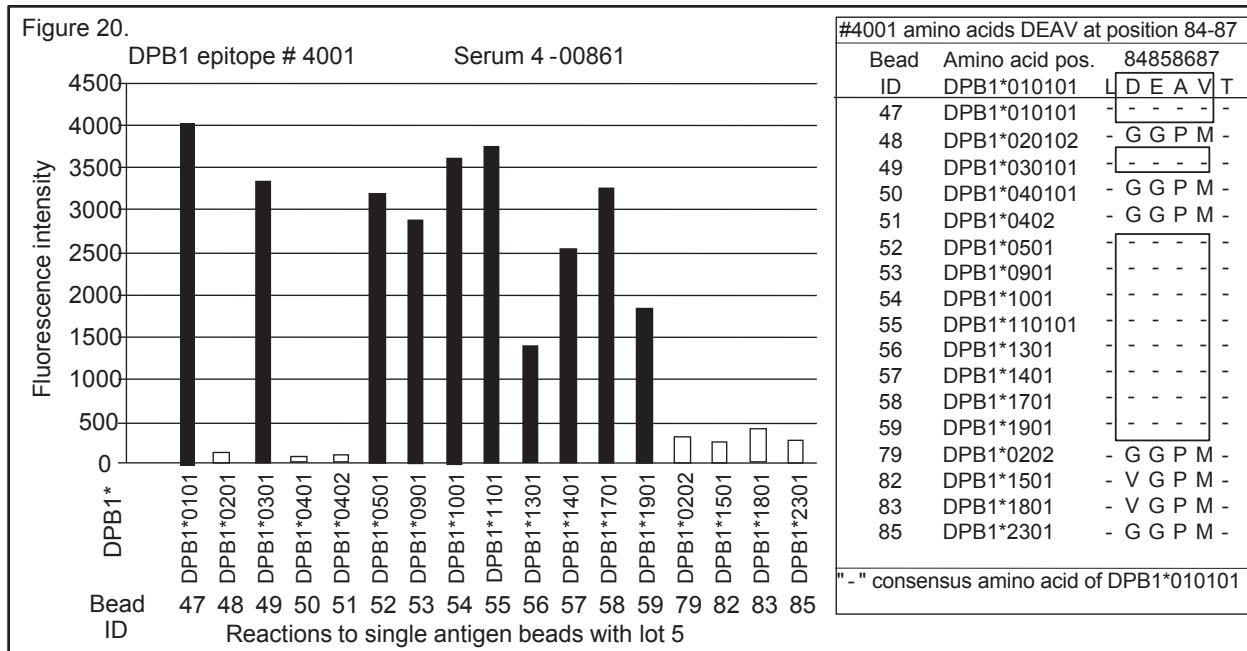
Figure 18.

Example of an epitope shared by DQB antigens and identified by a combination of two aa. Antigens DQ8(3) and DQ9(3) share the aa glycine (G) and proline (P) at positions 45 and 55 respectively on the HLA DQB chain. Therefore these two aa identify the epitope (Epitope # 2012, Table 8).

**Figure 19.**

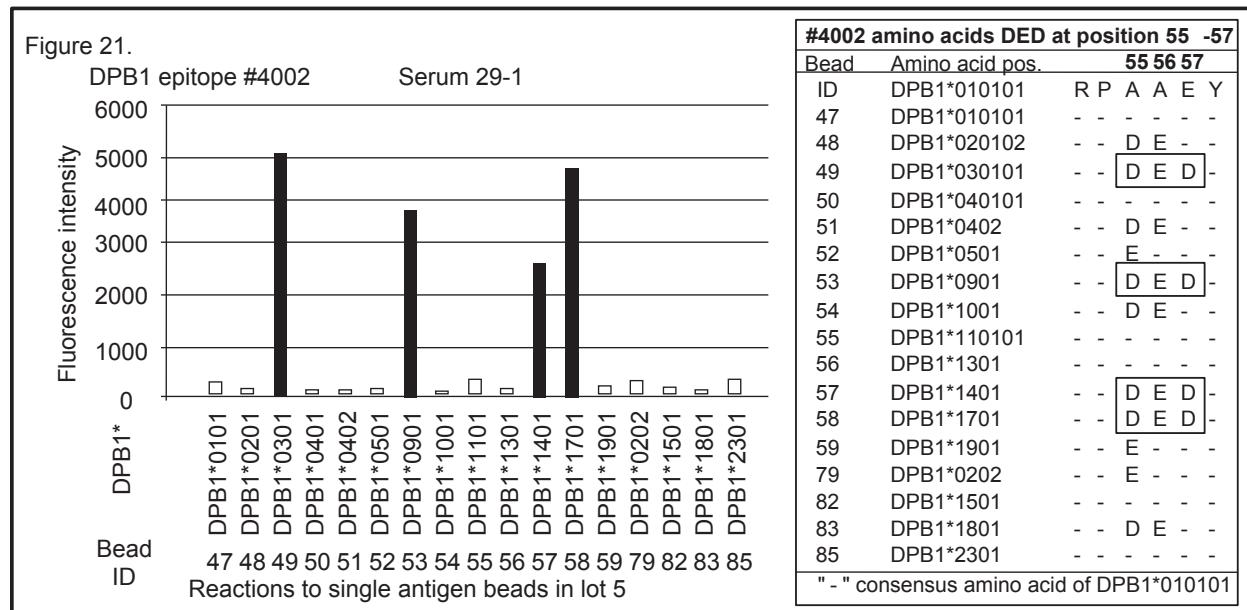
Example of a DQA epitope. The epitope defined by the aa lysine (K), histidine (H) and leucine (L) on the alpha chain of the DQA2 antigen. The three aa, in combination, define the epitope. (Epitope# 2017, Table 8).



**Figure 20.**

Solid bars indicate the positive reactions of serum 4-00861 (with a DPB1 #4001 pattern) to DP single antigen beads, listed by number, with each bead's DPB1 allele. Open bars indicate the

negative reactions to the beads that do not have the positive DPB1 antigens. In the accompanying sequence table, the amino acids and their positions are shown for each bead's DPB1 allele. The unique amino acids DEAV at positions 84 to 87 are the proposed epitope site of DPB1 #4001 (Table 9).

**Figure 21.**

Solid bars indicate the positive reactions of serum 29-1 (with a DPB1 #4002 pattern) to DP single antigen beads, listed by number, with each bead's DPB1 allele. Open bars indicate the

negative reactions to the beads that do not have the positive DPB1 antigens. In the accompanying sequence table, the amino acids and their positions are shown for each bead's DPB1 allele. The unique amino acids DED at positions 55 to 57 are the proposed epitope site of DPB1 #4002 (Table 9).

MICA Epitopes

Seven MICA epitopes have been defined to date (Table 11). All of these can be defined by a single aa. For example, epitope 6002—shared by the MICA*001, 002, 004, 007, 009, 012, 018, and 027 antigens—is defined by the aa glutamine (Q) at position 91 of the MICA molecule (Fig. 22). In two cases, the sera were absorbed by rMICA cell lines, the eluted antibodies reacting exclusively with certain MICA antigens. One antibody eluted from MICA*018 reacted exclusively with MICA*001, 012

and 018 antigens on the MICA SA beads. Those three MICA antigens exclusively share the aa threonine (T) at position 24, that aa defining epitope 6001. Another antibody eluted from the MICA*004 rMICA cell line reacted with MICA*027, 004 and 009 antigens, which share the aa tyrosine (Y), valine (V) or glutamic acid (E) at positions 36, 129 and 173, respectively. Any one of the aa defines epitope 6004 (Fig. 23).

Table 11. Seven MICA epitopes.

Epitope #	Single antigen beads reactive with eluted antibody	Position and unique aa for possible epitope ^a Sites	Alloserum / mAbs Tested	rMICA Cells used for adsorption
6001	MICA*001, 012, 018	(24T)	URUALB77	MICA*018
6002	MICA*001, 002, 004, 007, 009, 012, 018, 027 ^b	91Q	ND	ND
6003	MICA*004, 009	122V	ND	ND
6004	MICA*027, 004, 009 ^b	36Y/129V/173E	USAKAM 056	MICA*004
6005	MICA*017	91R	ND	ND
6006	MICA*004	181R	ND	ND
6007	MICA*027 ^b	213I / 251R	ND	ND

Columns, left to right, show: Epitope #; MICA antigens exclusively sharing the epitope; amino acid (including alternatives) that define the epitope; serum or monoclonal antibody; and for three allo sera the recombinant MICA cell lines used for absorption and elution of antibodies ND = Not done.

^aPossible alternative epitopes are separated by "/". Epitopes that are defined by more than a single position/aa are separated by "+". Amino acids that are not exposed at the surface of the HLA molecule are between parentheses.

^bMICA*019 also share the amino acid(s) shown. Due to higher than acceptable background fluorescence with the MICA*019 beads, it is not listed here.

Figure 22.

Example of a MICA epitope (#6002) defined by the amino acid glutamine (Q) at position 91 in the MICA antigen. This epitope is shared by the MICA*001, 002, 004, 007, 009, 012, 018, and 027 antigens. The 3D figure shows the amino acid is exposed at the surface of the molecule and therefore can bind the antibody (Table 11).

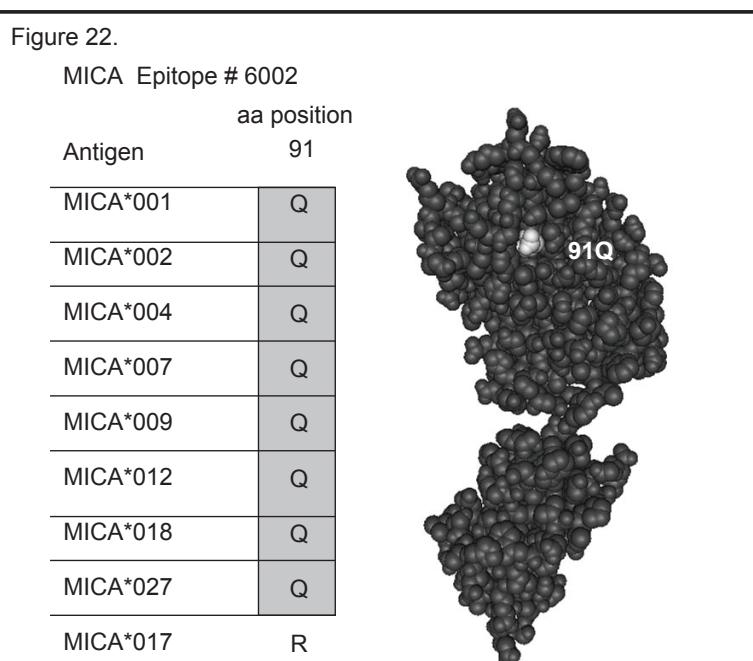
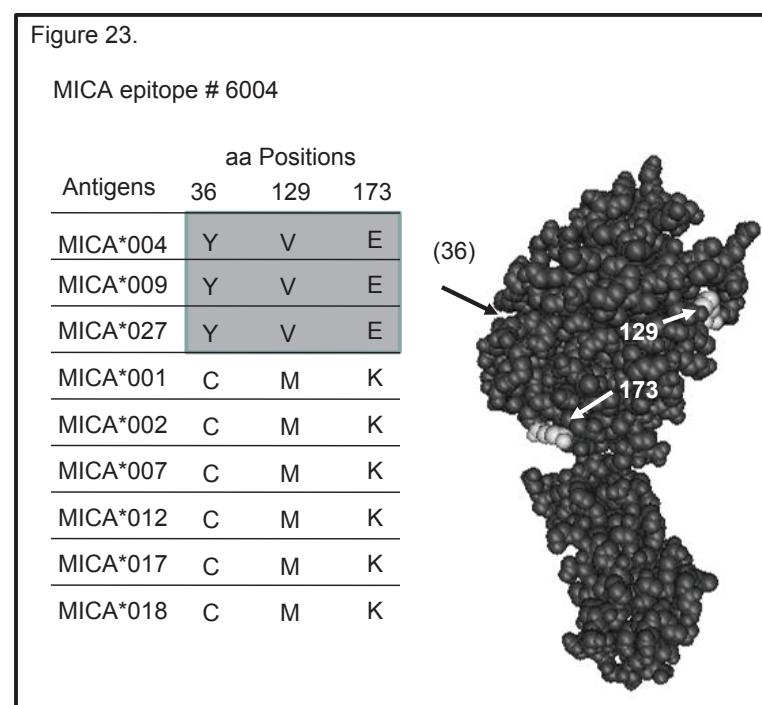


Figure 23.

MICA epitope defined by the aa tyrosine (Y), valine (V), and glutamic acid (E) at positions 36, 129 and 173, respectively. Epitope # 6004 is shared by MICA*004, 009 and 027 exclusively (Table 11).

**SUMMARY**

This chapter presents lists of HLA epitopes that have been defined to date. It also presents examples of reactions of mAb and eluted allosera with the class I, class II and MICA single antigen beads.

To date, we have identified 110 class I epitopes, of which 47 were defined by mAbs and 63 by alloantibodies that were eluted from rHLA class I single antigen cell lines. We listed 34 epitopes shared by the HLA-A locus antigens, 44 epitopes shared by HLA-B locus antigens, 4 epitopes shared by HLA-C locus antigens, 20 inter-locus epitopes shared by HLA-A-B locus antigens, 5 inter-locus epitopes shared by HLA-

B-C locus antigens and 3 inter-locus epitopes shared by HLA-A-B-C locus antigens.

Sixty HLA-DR epitopes have been defined mostly by one amino acid (aa) residue on the HLA-DR beta chain. Eighteen HLA-DQ epitopes have been defined on the HLA-DQB chain and on the HLA-DQA chain of the HLA-DQ antigens. A few DQ epitopes were defined by one aa residue. However, most can be defined by several alternative combinations of aa residues. DQA and DP epitopes—few in number at this time—were identified.

Only seven MICA epitopes have been defined to date. All epitopes can be defined by an exclusive amino acid.

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